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Marine mollusk thanatocoenoses along the coasts of the San Pietro Island (South-Western Sardinia): a first reasoning on species composition and biodiversity

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SUMMARY

We conducted a study on the taxonomic composition and diversity of thanatocoenoses of marine mollusks sampled at five sites on a circum-Sardinian island (San Pietro Island, South-Western Sardinia). The aim was to measure the differences in terms of biodiversity by analyzing shells >2 mm. We collected 71 taxa (level of diversity γ) at five different sites. Four species represented more than half of the sample: *Bittium reticulatum*, *Glans trapezia*, *Lucinella divaricata* and *Truncatella subcylindrica*. We observed a high difference in the number of taxa (level of diversity α) between sites (range: 18-41; after normalization using the Margalef index: 9.45-15.84). The turnover of β-diversity between low-energy sites was lower than that among high-wave energy sites; low-energy wave sites located near shallow and extensive *Posidonia oceanica* beds showed the highest values of diversity metrics. Climatic factors related to wave energy (exposure to prevailing West winds) and the presence of *Posidonia oceanica* beds could explain the diversity patterns. Although our diversity analysis conducted across mollusk thanatocoenoses may show some methodological weaknesses (the storage and accumulation time of the shells; the uncertainty regarding the taxonomy based exclusively on shells and the relative importance of the living populations in front of the sampling sites), we suggest that this "proxy" approach could allow for a rapid first assessment of the conservation status of coastal and marine ecosystems (i.e. Posidonion oceanicae habitat) when sampling time is short and there are several areas to explore.

INTRODUCTION

Thanatocoenoses, obtained classifying shells of dead mollusks, recently deposited as debris on sandy beaches or deep bottom sediments, may approximately represent the local fauna, and therefore a surrogate for the purpose of rapid assessment at α-diversity level (Warwick and Light 2002, Smith 2009). Furthermore, when comparing set of mollusk assemblages from different sites it is plausible to obtain fine-grained information also at β-turnovers and γ-diversity levels. Several authors have suggested that thanatocoenoses in marine intertidal habitats are sufficiently representative of local/seascape biodiversity to use in rapid, comparative assessments at different temporal and spatial scales, with implication for monitoring and conservation activities (Warwick and Light 2002, Kidwell 2007, Battisti and Cerfolli 2016, Cesarini et al. 2022).

Studies on mollusk assemblages mainly focus on living assemblages (Occhipinti-Ambrogi et al. 1988, Elias 1992, Terlizzi et al. 2005, Neira et al. 2014). This cannot be said for thanatocoenoses: the number of studies describing the biodiversity of recent mollusk thanatocoenoses is still low (e.g., Weber and Zuschin 2013, Battisti and Cerfolli 2016). Despite some methodological weaknesses that have already been highlighted (Warwick and Light 2002), the thanatocoenoses of mollusks – a “ghost” of the local biodiversity – has been proposed as a “surrogate tool” to evaluate species composition and diversity and turnovers of these invertebrates in coastal and marine sites of conservation interest (Powell et al. 1984, Powell et al. 1986, Smith 2008). Among the methodological weaknesses, highlighted by the literature, there are: (i) the storage and accumulation time of the shells which can be considered a confounding factor (Kidwell 2013). In this sense, the numbers of specimens of a single species are not reliable as an indicator of frequency and dominance; (ii) there is an uncertainty regarding the taxonomy based exclusively on shells and (iii) the analysis does not take into account the richness of the living populations in front of the sampling sites. In this sense, since anthropogenic alteration is a persistent and growing problem in coastal and marine ecosystems, the analysis of dead assemblages of mollusks could prove to be a useful, relatively rapid, and low-cost tool for detecting environmental changes in progress (Ciccolella and Bello 2006, Ferguson 2008).

Here, we carried out a study on the composition and diversity metrics (α-, β- and γ-levels) and evenness of different mollusk thanatocoenoses sampled in five sites located along the coast of a circum-Sardinian island (San Pietro Island, Sulcis Archipelago, South-Western Sardinia; Occhipinti-Ambrogi et al. 1988).

Carrying out a β-diversity approach (Magurran 2004), we hypothesized that the thanatocoenoses of mollusks sampled in coastal sites, dominated by high-energy winds, may show less diverse associations, when compared with analogous thanatocoenoses sampled in low energy sites where there is a large coverage of *Posidonia oceanica*.

MATERIALS AND METHODS

STUDY AREA

We carried out the study on San Pietro Island (Sardinia, central Mediterranean Sea), a circum-Sardinian island (11 km off the South-Western coast; WGS84 coordinates 39°08'N, 8°18'E) facing the Sulcis peninsula, with 51 km^2 in size (the sixth largest island of Italy; Fig. 1).

The island is of volcanic origin (Cioni and Funedda 2015). Since the high eco-biogeographic interest and conservation concern (e.g., Arrigoni and Bocchieri 1996, Bocchieri 2001, Battisti 2018, Battisti et al. 2021), the San Pietro Island is a Special Area of Conservation (S.A.C., Code “ITB040027 Isola di San Pietro” sensu Natura 2000 network of 92/43/EU Directive).

The different coastal and marine habitat types are listed in the management plan of the S.A.C., including the area occupied by *Posidonia oceanica* beds (size area: 1443 ha; Soriga et al. 2015). These are biocoenoses rooting at the bottom of the coastal areas, present in shallow and polyhaline waters which form submerged meadows in contact with photophilous phytocoenoses as Cystoserietalia and Caulerpetalia and with those sciaphilous of the order Rhodymenietalia (Soriga et al. 2015).

Among the main geomorphogenetic agents are the coastal processes (mainly winds and waves) which have produced various erosion processes depending on the exposure to the prevailing wind (Mistral, 65% of the events over the year). There is a sector facing north and west characterized by coves and a sector facing south characterized by beaches (Di Gregorio et al. 2010). In particular, the continental shelf in the north-western sector of the island is characterized by an irregular geomorphology with large outcrops of the Tertiary volcanic-sedimentary succession. The boundary between the inner and outer platform is represented by a series of cliffs dug into the volcanic substrate (Cau et al. 2016). The climate is typically Mediterranean (Tomaselli et al. 1973). The five sampling sites, distributed along the coast of the entire island, are divided as follows: three in sandy coastal areas and two in the relatively deep reef, on debris accumulated on the seabed perpendicular to the reef itself. Sample collection was performed by standardizing the collection in both sandy and detrital areas. At all sites, 20 collection points were randomly identified and 100±10 g of wet weight debris sieved for each point (using a field balance). The material collected in sandy coastal areas was collected using fine sieves (diameter 1x1 mm) and numbered and initialed plastic bags. The material collected on the reef was collected on the seabed by underwater activities, using a small boat and underwater operators. The deep-collected material was collected, as done in the sandy areas, using 1x1 mm sieves and initialed and numbered plastic bags. The interaction between rocky coast and prevailing wind has not been studied. For meteorological information present in Sardinia in August 2016, the Monthly Meteorological and Agrometeorological Summary - August 2016 (sar.sardegna.it) was also consulted.

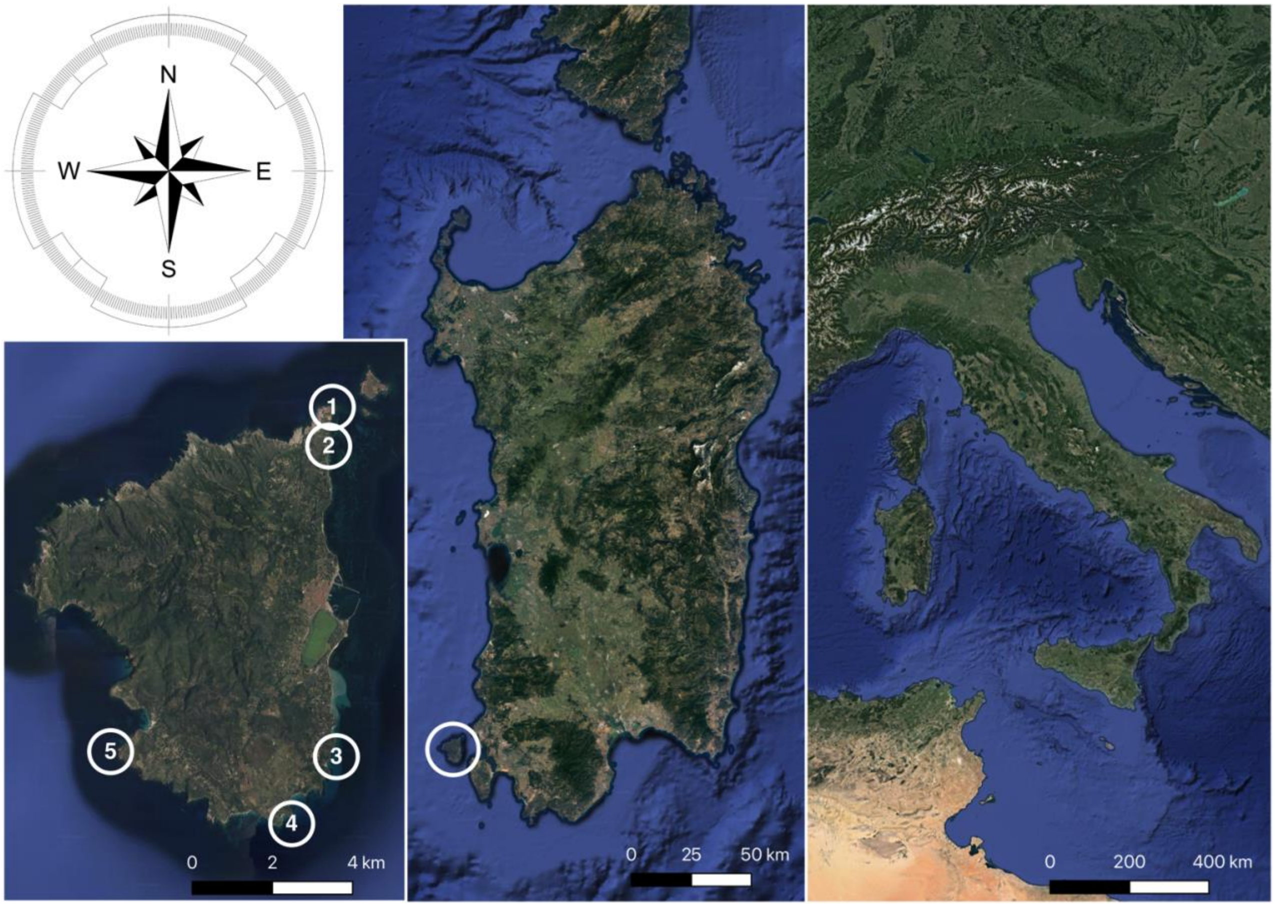

Figure 1. The study area (San Pietro Island, Sardinia, Italy). From n. 1 to 5: sampling sites (see study area for details).

SAMPLE COLLECTION

We selected five coastal sites (three beaches and two rocky coves) located, with different cardinal exposition, along the coastline of the San Pietro Island: (1) la Punta cove (39°11'6.60"N, 8°18'31.73"E); (2) Tacca Rossa beach (39°9'33.58"N, 8°18'23.39"E); (3) Girin beach (39°6'55.46"N, 8°18'32.75"E); (4) Bobba beach (39°5'39.75"N, 8°17'28.59"E); (5) Mezzaluna cove (39°5'58.02"N, 8°16'8.98"E; Fig. 1).

The five marine coastal sites are subdivided in two groups using the criterion of the wave energy and water depth (Vicinanza et al. 2011, Liberti et al. 2013, Carillo et al. 2016). Sites 1 and 5 (coves) are located along the rocky coast, respectively exposed to North and West and characterized by a high wave and wind energy; the beach sites 2, 3, and 4 are in context of low wave energy with an East and South wind exposition (Fig. 2). The sea bottoms exposed to North and West (sites 1 and 5) are deeper (depth: - 10 and -30 m) than the sea bottoms located in front of sites 2, 3, 4 where large *Posidonia oceanica* beds are present (< 10 m; Di Gregorio et al. 2010).

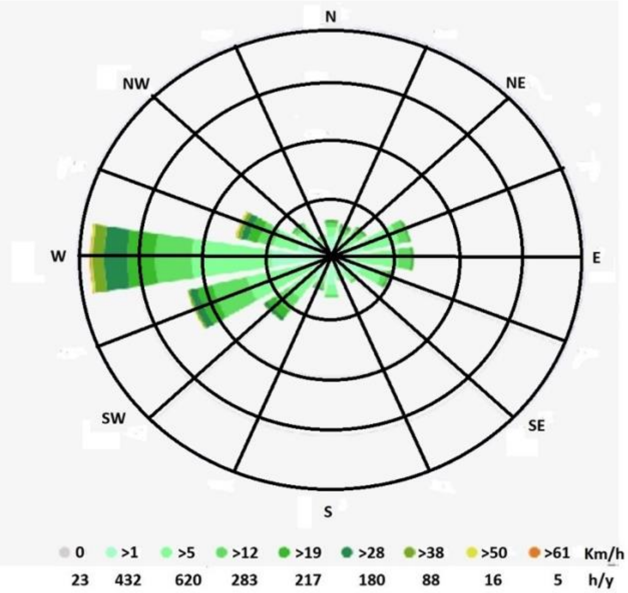

Figure 2. Wind rose of San Pietro Island (August 2016, in: www.meteoblue.com).

In each sandy site, we applied a randomized sampling design (Sutherland 2000), performing replicated mollusk collection points (n = 20), each one independent from another longitudinally located along the beach from the shoreline to the berm of storm. Between 20 and 30 of August 2016, we collected the mollusk debris using a rigid sieve of 20 cm width with 1x1 mm mesh opening size (max √2 diagonal). The debris has been gathered up to a depth of 20 cm. For the sampling of live benthic macroinvertebrates sieves (with a 250 µm - 0.5 mm mesh) were usually used: we have adopted sieves with a 1.0±0.1 mm mesh for samples of dead shells as the samples ≤ 1 mm were found to be difficult to classify due to having been subjected to abrasion and fragmentation.

At each sieving session, sand, debris and mineral fragments were removed and all mollusk shells (chitons, gastropods and bivalves) with dimensions > 2 mm were collected. For the two deep detrital seabeds, in front of the vertical rocky walls, along the coast, the thanatocoenoses were determined by adopting the same criteria.

As there is currently no universally accepted definition for the upper limit of the size range for micro-mollusks, and they are generally defined as mollusks no larger than 5 mm (Geiger et al. 2007, Geiger and Ruthensteiner 2008), for the purpose of this work we have considered the sampled shells of dead mollusks > 2 mm. Each debris sample, taken at a maximum depth of 20 cm, was sieved and allocated in a plastic bag (100 plastic bags in total, 20 bags for 5 sampling sites). The volume of each bag was 500 ml and the mean weight of the sampling debris was 100 ± 10 g (weighed with a field balance). Equivalently, the same was applied for bottom debris. In the laboratory we performed a taxonomic diagnosis at species level only for gastropods and bivalves. We excluded from the analysis a species of chiton, *Chiton olivaceus* (Spengler 1797). For the taxonomic diagnosis of gastropods, we referred to Giannuzzi-Savelli et al. (1994, 1996, 1999, 2003 and 2014) and for bivalves in the works of Huber (2010, 2015). After the collection of the shells, the specimens were each placed in separate containers (Smith 2008, Battisti and Cerfolli 2016). We considered specimens of intact shells of gastropods and intact shells of recently stranded bivalves. We have not considered shells that are more than 50% damaged in size. We have adopted the criterion of classification of the bivalves, with the two valves still attached, in accordance with the available bibliography (Schiaparelli 2008), and of assignment of the taxonomic unit according to the ICZN protocols where admissible as a type bearing a name is an animal or any part of an animal (ICZN Art. 72.5.1), (https://www.iczn.org/iczn/index.jsp).

For nomenclature, we used the checklist of Mediterranean marine mollusks (Bedulli et al. 1995a, Bedulli et al. 1995b, Oliverio 2008, Relini 2008, Schiaparelli 2008) and WoRMS (http://www.marinespecies.org) webpage.

DATA ANALYSES

We focused our analysis on the two most representative subgroups (Bivalvia and Gastropoda). For each taxon, we obtained the presence of species site by site regardless of their abundance. Although the number of specimens for each species was obtained, we did not consider this data in subsequent analyses, due to its poor representativeness as an indicator of true abundance (see Warwick and Light 2002, Ferguson 2008).

At assemblage level, we calculated the number of taxa (S, a measure of α-diversity at site-scale) and the normalized richness index (Margalef index, calculated as

$$Dm = S - 1/\log N$$

where S is the number of species and N the total number of specimens recorded in any site; Magurran 2004, Magurran and McGill 2011). Margalef index has been largely exploited to quantify the level of richness of living and dead mollusk assemblages (e.g., Zuschin and Piller 1997, Salas et al. 2006).

Moreover, we calculated the β-turnover index of Wilson and Shmida (1984), calculated between adjacent sites, as $\beta = [g(S) + l(S)]/2\alpha mean$, where g(S) is the number of taxa gained (i.e., newly recorded) and l(S) is the number of taxa lost between sites. The αmean corresponds to the average taxa richness between compared sites (where taxa richness is a measure of α-level diversity, *sensu* Whittaker 1965).

Finally, we obtained a total value of number of species, as a measure of γ-diversity (Magurran 2004). We used also the χ^2 robust test to compare the relative frequency of species (Dytham 2011). For data analyses, we used the SPSS 13.0 software (SPSS Inc. 2003). The alpha (α) was set at 0.05 level.

RESULTS

Totally, we collected 1325 specimens belonging to 71 taxa (γ-diversity level): 560 (42.26%) belonging to 29 taxa of Bivalvia and 763 (57.59%) belonging to 41 taxa of Gastropoda (Table 1). We also recorded two specimens of Chitonidae, *Chiton olivaceus* (Spengler 1797), not included in analyses at assemblage level.

Table 1. Checklist of taxa in the 5 sites in the San Pietro Island. •: occurrence. 1: la Punta cove; 2: Tacca Rossa beach; 3: Girin beach; 4: Bobba beach; 5: Mezzaluna cove (see Fig. 1).

Scientific name	1	2	3	4	5	Scientific name	1	2	3	4	5
Bivalvia						*Neverita josephinia* (Risso. 1826)			•		
Cardiita						*Rissoa auriscalpium* (Linnaeus. 1758)		•			•
Acanthocardia paucicostata (G. B. Sowerby II. 1834)		•	•			*Rissoa variabilis* (Megerle von Mühlfeld. 1824)		•			•
Acanthocardia (Rudicardium) tuberculata (Linnaeus. 1758)			•	•		*Rissoa ventricosa* (Desmarest. 1814)					•
Donax striatus (Linnaeus. 1767)		•		•		*Rissoa violacea* (Desmarest. 1814)		•			•
Donax trunculus (Linnaeus. 1758)		•	•	•		*Truncatella subcylindrica* (Linnaeus. 1767)		•			•

Donax venustus (Poli. 1795)		•			•
Parvicardium scriptum (Bucquoy. Dautzenberg and Dollfus. 1892)		•		•	
Carditida					
Cardites antiquatus (Linnaeus. 1758)		•			•
Glans trapezia (Linnaeus. 1767)		•	•	•	•
Cardita calyculata (Linnaeus. 1758)	•	•	•		•
Anomia ephippium (Linnaeus. 1758)			•	•	•
Arcida					
Arca noae (Linnaeus. 1758)		•		•	
Barbatia barbata (Linnaeus. 1758)		•	•	•	•
Striarca lactea (Linnaeus. 1758)	•				
Imparidentia					
Bornia sebetia (O. G. Costa. 1830)					•
Chama gryphoides Linnaeus. 1758		•	•		•
Donacilla cornea (Poli. 1791)		•	•		•
Pitar rudis (Poli. 1795)					•
Spisula subtruncata (da Costa. 1778)			•	•	
Ruditapes cfr. *decussatus* (Linnaeus. 1758)		•	•		
Moerella pulchella (Lamarck. 1818)			•		
Venerida					
Chamelea gallina (Linnaeus. 1758)		•	•		
Irus irus (Linnaeus. 1758)		•		•	
Venus verrucosa (Linnaeus. 1758)	•	•			
Myida					
Corbula gibba (Olivi. 1792)		•			•
Limida					
Limaria loscombi (G. B. Sowerby I. 1823)				•	
Lucinida					
Lucinella divaricata (Linnaeus. 1758		•	•	•	•
Mytilida					
Mytilus galloprovincialis (Lamarck. 1819)				•	
Ostreida					
Ostrea cfr. edulis (Linnaeus. 1758)				•	
Ostrea stentina (Payraudeau. 1826)	•		•		
Gastropoda					
Littorinimorpha					
Alvania cimex (Linnaeus. 1758)		•			•
Alvania discors (T. Allan. 1818)					•
Pusillina inconspicua (Alder. 1844)		•			•
Barleeia unifasciata (Montagu. 1803)		•			•
Melarhaphe neritoides (Linnaeus. 1758)	•	•			•
Caenogastropoda					
Bittium reticulatum (da Costa. 1778)	•	•	•	•	•
Cerithium alucastrum (Brocchi. 1814)		•	•		•
Cerithium vulgatum (Bruguière. 1792)	•	•			
Neogastropoda					
Pollia dorbignyi (Payraudeau. 1826)	•				
Columbella rustica (Linnaeus. 1758)	•	•	•		
Conus ventricosus (Gmelin. 1791)	•	•			•
Cyclope pellucida (Risso. 1826)		•	•	•	•
Gibberula miliaria (Linnaeus. 1758)		•		•	•
Hinia costulata (Brocchi. 1814)	•				
Pusia ebenus (Lamarck. 1811)	•		•		•
Mitrella scripta (Linnaeus. 1758)	•	•			
Pusia tricolor (Gmelin. 1791					•
Tritia mutabilis (Linnaeus. 1758)			•		
Trochida					
Clanculus corallinus (Gmelin. 1791)		•	•		•
Clanculus cruciatus (Linnaeus. 1758)	•	•			•
Steromphala divaricata (Linnaeus. 1758)	•				
Steromphala varia (Linnaeus. 1758)	•				
Steromphala umbilicaris (Linnaeus. 1758)	•		•		
Jujubinus exasperatus (Pennant. 1777)					•
Jujubinus gravinae (Dautzenberg. 1881)					•
Tricolia cfr. *pullus* (Linnaeus. 1758)		•			•
Lepetellida					
Diodora gibberula (Lamarck. 1822)			•		
Emarginula elongata (O. G. Costa. 1829)		•	•		•
Fissurella nubecula (Linnaeus. 1758)		•		•	•
Haliotis tuberculata (Linnaeus. 1758)				•	
Patelloidea					
Patella pellucida (Linnaeus. 1758)				•	
Patella caerulea (Linnaeus. 1758)	•	•	•	•	•
Pyramidelloidea					
Megastomia conoidea (Brocchi. 1814)					•
Lottioidea					
Propilidium exiguum (W. Thompson. 1844)					•
Caenogastropoda					
Monophorus perversus (Linnaeus. 1758)		•			•
Polyplacophora					
Chitonida					
Chiton olivaceus (Spengler. 1797)					•

Only four species significantly accounted for more than half of the sample (55.8%; χ^2 = 35.798, p <0.001): *Bittium reticulatum* (da Costa 1778) (n=338), *Glans trapezia* (Linnaeus 1767) (n=220), *Lucinella divaricata* (Linnaeus 1758) (n=110) and *Truncatella subcylindrica* (Linnaeus 1767) (n=72).

Totally, Margalef index showed a total value of 22.42 (sub-assemblages: 13.88 for Gastropoda and 10.19 for Bivalvia). Analyzing the differences between sites, we observed a high difference in number of total taxa ranging from 18 (site 1: La Punta) to 41 (both, site 2: Tacca Rossa and 5: Mezzaluna). After normalization, Margalef index ranged from 9.45 (La Punta) to 15.84 (Mezzaluna; Table 2).

We obtained an interesting pattern in diversity metrics: (i) two sites (n. 3: Girin beach and n. 4: Bobba beach) showed an intermediate normalized species richness (between 10 and 13); (ii) two sites (n. 2, Tacca rossa beach and n. 5: Mezzaluna cove) showed the highest normalized species richness (>13); (iii) a site (n. 1: La Punta) showed the lowest value both of normalized species richness.

The β-turnover index showed high values (≥ 0.5) between adjacent sites (Table 2). However, the extreme high energy sites (coves n. 1 and n. 5) showed the highest values of species turnover when compared with the low wave energy sites (beaches n. 2, 3, and 4). These last showed a higher similarity (low turnover) among them (≤ 0.5).

Table 2. Diversity metrics for the set of mollusk thanatocoenoses sampled at San Pietro Island (Sardinia, Italy). N: number of specimens; S (α): number of species (a measure of α-diversity at site-scale); Dm: Margalef normalized species richness index; β: Wilson and Shmida's β-turnover index between adjacent sites. 1: la Punta cove; 2: Tacca Rossa beach; 3: Girin beach; 4: Bobba beach; 5: Mezzaluna cove (see Fig. 1).

<table>
<tr><th>sites</th><th colspan="2">1</th><th colspan="2">2</th><th colspan="2">3</th><th colspan="2">4</th><th colspan="2">5</th><th colspan="2">tot</th></tr>
<tr><td>N</td><td colspan="2">63</td><td colspan="2">745</td><td colspan="2">113</td><td colspan="2">69</td><td colspan="2">335</td><td colspan="2">1325</td></tr>
<tr><td>S (α)</td><td colspan="2">18</td><td colspan="2">41</td><td colspan="2">27</td><td colspan="2">21</td><td colspan="2">41</td><td colspan="2">71</td></tr>
<tr><td>Dm</td><td colspan="2">9.45</td><td colspan="2">13.93</td><td colspan="2">12.66</td><td colspan="2">10.88</td><td colspan="2">15.84</td><td colspan="2">22.42</td></tr>
<tr><td rowspan="2">β</td><td></td><td colspan="2">0.661</td><td colspan="2"></td><td colspan="2">0.583</td><td colspan="5"></td></tr>
<tr><td colspan="3"></td><td colspan="2">0.500</td><td colspan="2"></td><td colspan="2">0.694</td><td colspan="3"></td></tr>
</table>

DISCUSSION

San Pietro Island's thanatocoenoses seem to be characterized from a relatively high richness (γ-diversity = 71 taxa) when compared to other analogous Mediterranean assemblages (e.g., along the Sicilian coasts: 55 taxa; Battisti and Cerfolli 2016).

We obtained a peculiar pattern among the five sites. Dead mollusk assemblages at sites with low wave energy appear to have the highest values of diversity metrics. These sites overlook extended and shallow *Posidonia oceanica* beds (Soriga et al. 2015). Comparing the thanatocoenoses of mollusks in high energy and low energy coastal zones, the turnover is higher in high energy ones. We have no ecological implications on this model but only marine meteorology and biophysical implications that could be better investigated in the future. The presence of the Posidonion oceanicae habitat type in shallow sites, characterized by areas with low wave energy, could explain the differences in species composition, diversity, and turnover (Cau et al. 2016, Di Gregorio et al. 2010). In this first exploratory approach, we used the data to induce hypotheses (i.e., the role of wave energy generated by the prevailing winds, the role of *Posidonia oceanica*) about the possible causal factors explaining the obtained models (inductive approach; Guthery 2007).

In consideration of the large number of species, of the strictly species-specific habitat relationships and of the consequent role of dead mollusks as an indicator of biodiversity and of the non-invasive sampling technique, the analysis of thanatocoenoses could represent a useful low-cost tool for the monitoring of the state of marine and coastal ecosystems in contexts of conservation crisis. In this regard, on San Pietro Island, urbanization and population growth may have been disturbing factors on the marine ecosystems (Pellerano and Rivano 1997, Pellerano 2001, Ruggeri 2003). The analysis of the effects of increasing anthropic pressure on the structural and functional parameters of marine and coastal ecosystems and the evidence of the consequent fragmentation of *Posidonia oceanica* beds (Bedini 2003, Giani and Cossu 2003, Milazzo et al. 2004, Riding et al. 2005, Bravo et al. 2016), are study perspectives that could be monitored using the approach of coarse-grained thanatocoenoses, albeit with all the limitations already highlighted in the literature (Martin 1999). Although the standardized collection of dead specimens, sampled along the coast, has the advantage of optimizing the time of field activities and reducing the sampling costs of live biological material, methodological weaknesses such as storage time and accumulation of shells can be a confounding factor (see Kidwell 2013). We consider the number of species not entirely reliable as an indicator of frequency and dominance. There remains uncertainty about the taxonomy based exclusively on dead shells. Furthermore, the analysis does not consider the richness of the populations in front of the sampling sites. Accepting the suggestions present in the literature (e.g., Warwick and Light 2002, Ferguson 2008), we believe that further planning efforts should be devoted to overcoming these weaknesses, to facilitate the rapid monitoring of complex marine and coastal ecosystems using the preliminary approach based on the thanatocoenoses (or taphonomy; Martin 1999) in conjugation with other techniques (i.e., DNA barcoding, eDNA, etc; Collins et al. 2018).

In this regard, our exploratory research has improved local knowledge of these neglected taxa in a poorly studied region, suggesting some possible investigative approaches.

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